A Revision of Certain Points in the Early Development of Peripatus capensis.

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With Plate 20.

Some valuable material of Peripatus capensis and Peripatus Balfouri was very kindly presented to the Zoological Laboratory of the Imperial College of Science by the widow of the late Prof. Adam Sedgwick, and at the suggestion of Prof. MacBride, who handed over the material to me, I have revised two points in Prof. Sedgwick's "Monograph of the Development of Peripatus capensis," which had raised a great deal of controversy when the paper was published.

I wish to express my most sincere thanks to Prof. MacBride for placing this material at my disposal and also for the kind assistance and very valuable advice which he has given me during my work.

General Remarks.

The first point I shall deal with is the theory propounded by Sedgwick that, in the early stages of Peripatus capensis and even as late as stage B, there are no cell limits (**10**).

This revolutionary statement caused a great deal of comment at the time, threatening to overthrow the prevailing idea that the ancestral Metazoon was a colonial Protozoon, and suggesting instead that it was a multinucleated Infusorian-like animal. It influenced all Sedgwick's later work, and in his paper on the "Inadequacy of the Cellular Theory of Development" (11) some years later, he says that his subsequent work has amply confirmed his earlier view that embryonic development "must be regarded as a multiplication of nuclei and a specialisation of tracts and vacuoles in a continuous mass of vacuolated protoplasm" (1883).

In this paper he deals with two tissues of the vertebrate embryo—the mesenchyme and the system of peripheral nerve trunks. The mesenchyme has always been described as consisting of branched cells lying between the ectoderm and endoderm. Sedgwick rejects the idea of cells here and affirms that the so-called mesenchyme consists of a reticulum with nuclei at its nodes, and that these nodes are what have always been described as cells. He also comes to the conclusion that nervous and muscular tissues are special developments of the same primitive reticulum, and, in fact, that all tissues are merely modifications of a reticulum.

The second point in Sedgwick's paper, which was responsible for a large amount of controversy, was with regard to the nephridium. He maintains that it is of a purely mesodermal nature, and that it opens directly to the exterior.

There have been repeated misunderstandings and misinterpretations of the term "nephridium." This name was given to the structures previously called "segmental organs," by Sir E. Ray Lankester in 1877. We may quote his original definition:

". . . in Rotifera, Flatworms, Gephyræa (not the genital ducts), Mollusca, in the metameres of Chætopoda, in the Vertebrata, and even in some Arthropoda we have evidence of the existence of a single pair of canals, more or less highly modified by glandular developments, which usually open by ciliated funnel-like mouths into the cœlome at one end and directly to the exterior in the neighbourhood of the anus, or into a cloacal chamber at their other end, thus placing the cœlome in communication with the exterior.

"This pair of ciliated funnels appears to be the same organ in all cases. Primarily it develops like the stomodæum by an ingrowth of the ectoderm or deron. At present no name is in use for this important pair of organs; they are spoken of as 'segmental organs' in some groups, as 'primitive excretory organs' in others. Since very usually these canals acquire an excretory function and give rise to kidneys, though they may also serve as genital ducts, I propose to call them by the diminutive of the Greek word for a kidney—namely, 'nephridium'" (**8**).

From this it will be seen that Lankester believed that the nephridia were most decidedly of ectodermal origin, and this view was generally accepted.

Meyer, who in 1886–7 studied the development of the Annelida carefully, came to the same conclusion with regard to the development of the nephridium (**9**). On the other hand, Bergh (1899) (**1**) and Bürger (1902) (**2**) maintained that the nephridium of the Oligochæta was of cœlomic origin. They said it developed as an outgrowth of the cœlome which fused with the ectoderm, and finally became hollowed out. But in 1895 Goodrich (**3**) proved that the nephridia of Oligochæts were of ectodermal origin.

They develop from large outer layer cells (funnel cells) and pass through a pronephridial stage.

"In the first (most forms) and sometimes in the trunk segments (Chætogaster) they never develop beyond that stage. In the other segments the nephridia grow towards and open into the cœlome by means of a funnel formed from the original 'funnel cell.'"

Goodrich also (1897–8) (**4**) studied the development of some marine Annelids and found that in all those which he examined the nephridia were of essentially ectodermal origin, consisting of blind tubes which are ectodermal ingrowths, and later on, acquire a communication with the cœlome. So-called nephridia, which develop from the cœlome, as in Terebellidæ and Mollusca, he terms "cœlomoducts," and he draws attention to the presence of both a cœlomoduct (the genital duct) and a nephridium in the same somite of Lumbricus, hence the two structures cannot be homologous.

The development of the *Alciopinæ*, also worked out by Goodrich in 1900 (**4**), is extremely interesting. In these worms the nephridia are closed internally, and are provided at the inner end with well-developed solenocytes. The genital funnel (cœlomostome) develops later, and, growing down, becomes grafted on to the nephridial duct, and, finally, an opening is formed at the point of junction. Hence the genital products pass down the nephridial duct to the exterior. This development was investigated in more detail by Goodrich in 1912 (**5**), and he found that the nephridium is a slender tube, and that the cœlomostome grows back as a pocket from the septum near the nephridium and unites with the nephridium at maturity.

Again, Staff in 1910 (**12**) found that in the Oligochæt *Criodrilus* the mother-cells of the nephridium appear in the ectoderm.

The nephridia of *Sipunculus* also have been found to appear as solid ectodermal ingrowths in which a cavity appears at a later stage. They eventually open into the cœlome, and the cells at the point of junction give rise to the funnel (**6**).

This overwhelming mass of evidence in favour of the ectodermal origin of the nephridium casts considerable doubt on the correctness of Sedgwick's conclusions, especially as at about the same time that Sedgwick was working out the development of *P. capensis* (**10**), Kennel was doing the same with *P. Edwardsii* (**7**), and he did not agree with Sedgwick in regard to the origin of the nephridium.

He declared that there was an ectodermal inpushing which ultimately became a canal, and that the funnel only of the nephridium was mesodermal. He also said that the funnel was in direct communication with the body cavity, but it is quite probable that this was due to the fact that the vesicle is very thin-walled and easily broken.

Sedgwick denied the existence of an ectodermal ingrowth of any importance.

"I have only to say that the ectodermal ingrowth at the opening of the nephridium is extremely inconspicuous, and that at the early stage immediately before and after the establishment of the external opening, an ectodermal part such as Kennel describes can only be made out with difficulty" (**10**, p. 81), and holds that the cœlome opens directly to the exterior at the base of the leg and just external to the nerve cord.

(i) Presence of Cell Limits in Peripatus capensis at Stage A.

The following statements are taken from Sedgwick's paper and relate to stage A of the embryo:

"The ectodermal part of the embryo consists of a closely-reticulated protoplasm which contains a single layer of oval nuclei of fairly uniform size" (**10**, p. 52).

"During stages A and B the ectoderm on the dorsal and ventral surfaces is composed of what may be called cubical cells, . . . but these cells are not isolated from one another or from the endoderm" (**10**, p. 53).

"With regard to the internal boundary of the ectoderm in the gastrula stage, there was no line of demarcation between it and the endoderm. In stage B the mesoderm appears, but causes no break in the continuity" (**10**, p. 55).

"We cannot speak of cells till . . . . stage B" (**10**, p. 106).

He describes the endoderm as the "inner portion of the vacuolated wall of the embryo. The two are perfectly continuous" (**10**, p. 65), and as being, at the close of stage E, "a layer of vacuolated protoplasm with nuclei of irregular size" (**10**, p. 66).

Considering these statements as a whole, I take it that Sedgwick denies the presence of cells altogether in the endoderm at this stage, but admits that there are "what may be called cubical cells" in the ectoderm. But he also says (**10**, p. 106) that we cannot speak of cells till stage B. By this I suppose he means that there are no cell-walls visible till that stage.

In examining sections of stage A, I found the general appearance practically that described by Sedgwick—the ectodermal nuclei oval and rather crowded together, the endodermal nuclei larger, very irregular in outline, and scattered in the protoplasm, which was extremely vacuolated, particularly on the dorsal side. I must admit that when one first sees sections of the early stages of P. capensis Sedgwick's theory seems quite probable.

But careful examination with a $\frac{1}{12}$-in. oil-immersion objective showed that in the ectoderm one could distinguish a certain number of undoubted cell-walls.[1] In some parts it was impossible to make out the cell-walls with certainty, partly owing to the crowding of the nuclei, and partly owing to the extremely large vacuoles on the dorsal side, which made it almost impossible definitely to distinguish cell-walls from strands of protoplasm. But those that could be distinguished were quite undoubtedly cell-walls. The existence of even a certain number of cell-walls makes it impossible for the ectoderm at this stage to be a "closely reticulated protoplasm with a layer of nuclei."

The cell-walls of the endoderm are much more difficult to distinguish, as they are very irregular and might be confused with protoplasmic strands, but, on very careful examination, one can make them out almost without exception.

Sometimes one finds what appears to be a cell without a nucleus, but this is explained by the large size of the cells, which makes it possible for a section to pass through the cell and yet not through the nucleus. Pl. 20, figs. 3–5 represent three sections drawn in series, and, allowing for the irregularity of the walls, one can see that they correspond with one another in all three sections. This would be rather unlikely if they were simply protoplasmic strands.

[1] By cell-wall I mean the layer of non-protoplasmic substance which is formed as a secretion and which separates the cells from one another. In preparations preserved and stained by the usual methods and examined under a high power of the microscope, it appears as a thin dark line.

The dividing line between ectoderm and endoderm is quite a well-defined thing, and can be seen relatively easily.

There seems to me to be not the slightest doubt of the presence of cell-walls at this stage of the development of P. capensis, although I do not mean to infer by this that I consider that each cell in a multicellular organism is absolutely independent of every other cell. There must be some connection, and that probably by protoplasmic strands. But to deny that there are cell-walls and cells, in the accepted sense of the word, in the earlier embryonic stages of Peripatus seems to me entirely wrong.

The difficulty in seeing them is due to the extremely large size and irregular shape of the cells. This is, no doubt, caused by the fact that the ovum of P. capensis is passing from the yolked to the non-yolked condition, and the yolk is bound to have distended the cells.

Two things, in all probability, accounted for the fact that Sedgwick did not admit the existence of cell-walls. One must take into account, first, the extremely large size and irregularity of the endodermal cells and the presence of numerous vacuoles; and second, the very poor preservatives that were available in his time.

(ii) The Origin of the Nephridium.

According to Sedgwick, the whole of the nephridium is mesodermal.

Each somite becomes divided into a dorsal and ventral part by a septum. The dorsal part eventually disappears, except in the posterior segments of the body, where it gives rise to the generative organs, but the ventral part gives rise to the nephridium. At stage F this consists of "(1) . . . a vesicular internal part extending to the hind end of the appendage and forwards as a blind diverticulum, and opening into (2) a tubular part projecting ventrally and opening to the exterior" (**10**, p. 76). "The tubular part is the nephridium and its opening into the vesicle is the funnel" (**10**, p. 79).

Kennel maintains that the tubular part which opens to the exterior is ectodermal and that the funnel only is mesodermal. But Sedgwick's reply to this was that the ectodermal ingrowth was too inconspicuous to be taken into account.

Kennel partly bases his views on the difference in the appearance of the ectodermal and mesodermal nuclei. The ectodermal nucleus he describes as "rundlich, bei Sublimatbehandlung homogen und färben sich nicht sehr intensiv. Die Kerne der mesodermzellen sind länglich, mehr körnig, stehen dichtgedrängt und färben sich in Pikrocarmin und Boraxcarmin sehr intensiv" (7, p. 39).

This differentiation of the nuclei is very well seen at an early stage, where the cavity of the somite is just being divided into a dorsal and a ventral part. The mesodermal nuclei look almost like a string of beads inside the ectoderm. The difference is not quite so marked in the later stages, but it is still evident. (I had some difficulty in staining these sections sufficiently without obliterating all the detail, and the result was that they were rather faintly stained. This is probably the reason why the difference between the two kinds of nuclei is not so clear as in the earlier stages.)

The ectodermal ingrowth described by Kennel is, without doubt, a very conspicuous thing and forms a part of the nephridium, as will be clearly seen from Pl. 20, fig. 8. The distinction between its nuclei and those of the funnel or the vesicle is well marked.

Pl. 20, fig. 6, shows the beginning of the ectodermal ingrowth and the ventral part of the somite. Pl. 20, fig. 7, is a later stage, probably just before the opening of the ectodermal canal into the cœlome, and Pl. 20, fig. 8, shows a much later stage in which one can distinguish the ectodermal part which has grown in, from the mesodermal part which originated from the ventral part of the somite.

The internal vesicle seems to me to be larger than is shown in Sedgwick's diagrams, and fills almost the whole cavity of the leg. Part of it is seen in surface view in Pl. 20, fig. 8, but the nuclei which I consider mesodermal extend farther down the leg, hence the vesicle also must extend farther down, although one cannot see the whole of its boundary.

Pl. 20, fig. 7, in particular seems to me to prove the existence of an ectodermal ingrowth without the slightest doubt. The ingrowth there is very conspicuous and it is most certainly ectodermal, even without the evidence of the nuclei.

If this ingrowth only formed part of the tube it would not be possible to say that it was a real homologue of the Annelid nephridium, as it might be merely a "gateway" to a cœlomiduct. But when, as in this case, the whole tube is ectodermal with the exception of the funnel, which is mesodermal, and when one considers that Peripatus is the most primitive Arthropod and most nearly allied to the worms, in which, as we have seen, the true nephridia are ectodermal, then I think one may say that the "nephridium" of Peripatus is not a cœlomiduct, but that it is homologous with the true nephridium of worms.

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EXPLANATION OF PLATE 20,

Illustrating Miss E. H. Glen's paper on "A Revision of Certain Points in the Early Development of Peripatus capensis."

List of Abbreviations.

app. Leg. *cav.app.* Cavity of leg. *c.b.s.* Central blood space. *ect.* Ectoderm. *ect.in.* Ectodermal ingrowth. *end.* Endoderm. *f.* Funnel. *l.b.s.* Lateral blood space. *neph.* Nephridium. *v.som.* Ventral part of somite. *v.n.c.* Ventral nerve cord. *w.v.som.* Wall of ventral part of somite grazed by razor.

[All the drawings were made with the aid of a Zeiss-Abbé drawing apparatus.]

Fig. 1.—Transverse section of an embryo of stage A, showing nuclei and cell-walls of ectoderm and endoderm.

Fig. 2.—Part of the same section as seen with a $\frac{1}{12}$-in. oil immersion objective.

Figs. 3–5.—A series of transverse sections of the same embryo, to show that the cell-walls occur in approximately the same position in all three sections.

Fig. 6.—Transverse section of an embryo of late spiral stage, showing the beginning of the ectodermal ingrowth and the slight down-growth of the somite.

Fig. 7.—A later stage where the ectodermal ingrowth is extending inwards, but does not yet open into the cœlome.

Fig. 8.—A still later stage where the ectodermal ingrowth has become a canal and now communicates with the cœlome.